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Synthesis of a Potent Vinblastine: Rationally Designed Added Benign Complexity

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Supporting Information

ABSTRACT: Many natural products, including vinblastine, have not been easily subjected to simplifications in their structures by synthetic means or modifications by late-stage semisynthetic derivatization in ways that enhance their biological potency. Herein, we detail a synthetic vinblastine that incorporates added benign complexity (ABC), which improves activity 10-fold, and is now accessible as a result of advances in the total synthesis of the natural product. The compound incorporates designed added molecular complexity but no new functional groups and maintains all existing structural and conformational features of the natural product. It constitutes a member of an analogue class presently inaccessible by semisynthetic derivatization of the natural product, by its late-stage functionalization, or by biosynthetic means. Rather, it was accessed by synthetic means, using an appropriately modified powerful penultimate single-step vindoline−catharanthine coupling strategy that proceeds with a higher diastereoselectivity than found for the natural product itself.

Vinblastine (**1**) and vincristine (**2**) were originally isolated from *Catharanthus roseus* (L.) G. Don[1,2] and are the most widely recognized members of the vinca alkaloids (Figure 1).

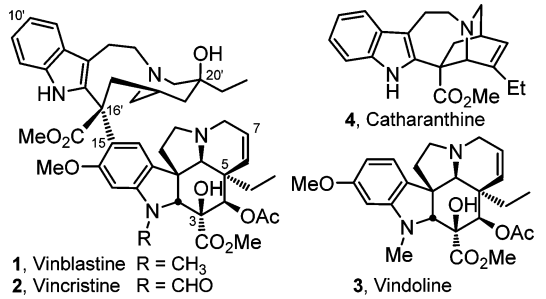

Figure 1. Natural products.

They have a rich history and were among the first natural products introduced clinically to treat cancer.[3] Their discovery defined an important antineoplastic mechanism of action, entailing the inhibition of mitosis via tubulin binding and inhibition of microtubule polymerization,[4] that today is regarded as one of the most successful drug targets in oncology. Although additional natural products have been shown subsequently to act on tubulin (e.g., taxol, epothilones, eleutherobin, dolastatins), vinblastine and vincristine differ in that they bind at the tubulin−tubulin head-to-tail dimer interface.[5] Because of their clinical importance, complex structures, and unique mechanism of action, vinblastine and vincristine have attracted extensive synthetic and mechanistic efforts since their original discovery.[3,6,7]

In recent studies, we described concise total syntheses of vinblastine and related natural products based in part on introduction of a powerful single-pot two-step diastereoselective Fe(III)-promoted vindoline/catharanthine coupling and subsequent Fe(III)−$NaBH_4$/air mediated in situ C20′ oxidation.[8,9] These developments have permitted systematic studies of the effects of deep-seated changes in the natural product structures within either the lower vindoline or upper catharanthine-derived velbanamine subunits.[9] Among the most significant of the observations made to date in these studies is that while removal of individual substituents or key structural components of the natural products typically results in reductions in biological activity,[8−14] addition of structural features can substantially improve biological properties.[15,16] As a result of our demonstration of the importance of the addition of a key indole C10′ substituent (10′-fluorovinblasine)[15] and with the discovery of the remarkable impact of select C20′ alcohol replacements (C20′ ureas),[16] it appeared that the spatial placement of the indole at one end of velbanamine and the C20′ ethyl group at the other are two especially important features of the structure. The X-ray structure of tubulin-bound vinblastine[5] (Figure 2) indicates that both fit into well-defined protein pockets on the tubulin α and β subunits, respectively, deeply embedded in the tubulin binding site with each occupying corners of a T-shaped bound conformation of vinblastine. The core of the velbanamine subunit fills the intervening space and serves as a rigid scaffold that fixes the placement of these two anchoring groups.

Herein, we report a compound, representative of a strategy rarely considered with complex natural products, where increasing the complexity of the core structure provided a stunning 10-fold enhancement in the biological potency. The results are sufficiently remarkable to suggest that even in instances where Nature appears to have optimized a natural product structure for activity, addition of even seemingly benign complexity to its underlying core structure may be possible that further and substantially improves on what Nature

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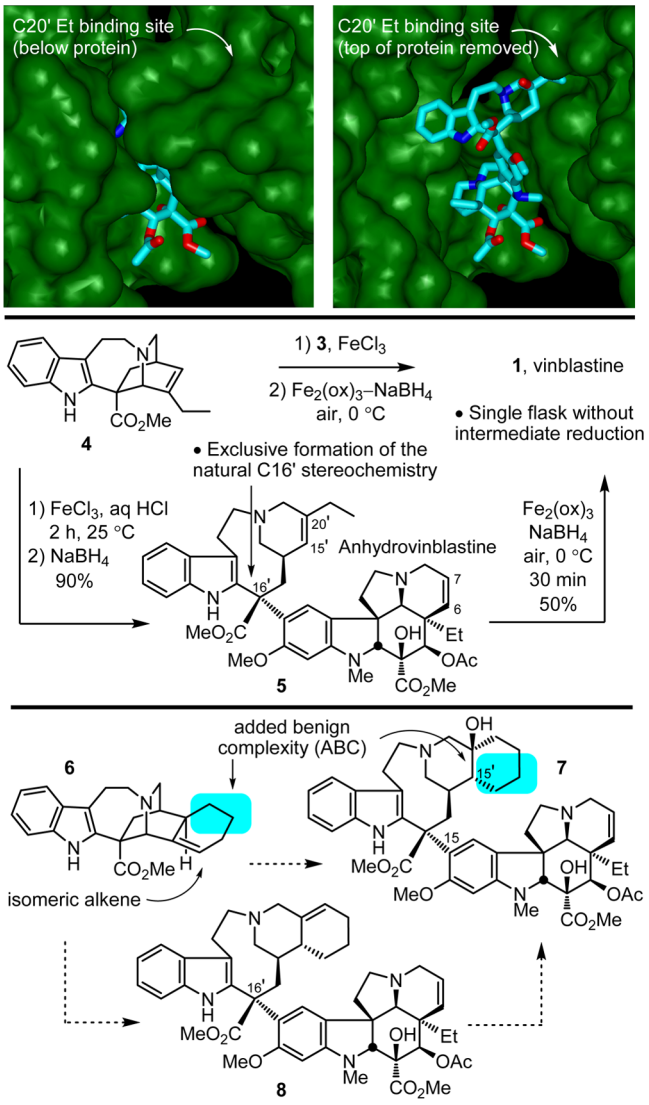
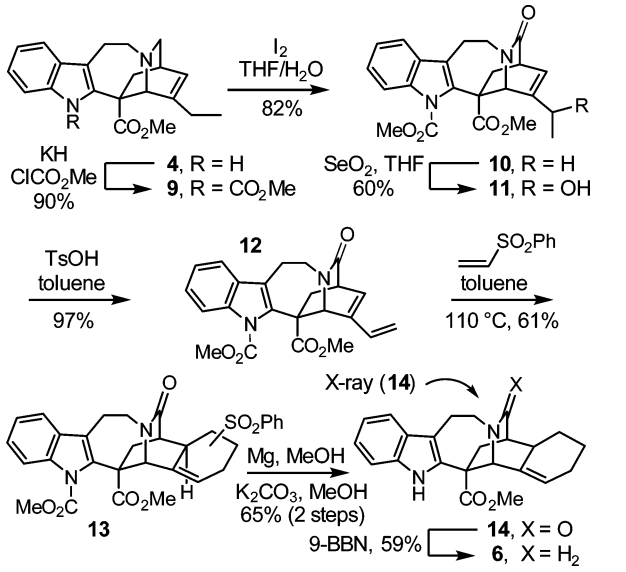


Figure 2. (top) X-ray crystal structure of tubulin-bound vinblastine (pdb 1Z2B)[5] highlighting the C20′ ethyl binding site at the dimer–dimer interface where vinblastine binds (left) and site of binding with top of proteins removed to visualize bound vinblastine (right). (bottom) Original protocol for accessing vinblastine and modified coupling and subsequent HAT oxidation for accessing **7**.

only started. For vinblastine and the work herein, this entailed incorporation of the C20′ ethyl group into an added C15′–C20′ cis-fused six-membered ring that further fills the C20′ ethyl binding pocket. Unlike molecular editing of structures by diverted synthesis,[17] the addition of a key functional group, derivatization of the natural product, tactics of simplification of complex biologically active natural products,[18] or introduction of conformational constraints,[19] the design of **7** entails introduction of added benign complexity (ABC) to the underlying core structure of vinblastine (two added sp^3 methylenes and a stereocenter) to more favorably occupy and fill a protein binding site without changing the intrinsic conformational or structural features of the natural product. Compound **7** represents a member of an analogue class inaccessible by semisynthetic derivatization of the natural product, by its late-stage functionalization, or by biosynthetic means but was prepared herein by late-stage divergent synthesis[20] based on the penultimate single-step vindoline–catharanthine coupling strategy. Its synthesis used **6**, bearing an alkene exocyclic (vs endocyclic) to the catharanthine skeleton, which incorporates the added fused six-membered ring and additional C15′ stereochemistry (Figure 2). This change in the substrate also substantially improved the diastereoselectivity (>6:1 vs 2:1) of the in situ alkene oxidation reaction used for introduction of the C20′ alcohol while maintaining the exclusive generation of the natural C16′ stereochemistry at the critical coupling site.

Following a known three-step synthesis,[21] intermediate **11** was prepared from the free base of catharanthine (**4**, $16/g) by indole protection (2.5 equiv of KH, THF, 0 °C; 2.5 equiv of $ClCO_2Me$, 0–23 °C, 15 h, 90%), single-step conversion of **9** to the lactam **10** (4.5 equiv of I_2, 9 equiv of Na_2CO_3, THF/H_2O, 0–23 °C, 22 h, 82%), and subsequent allylic oxidation (2 equiv of SeO_2, THF, 65 °C, 18 h, 60%) under modified reaction conditions (THF vs EtOH) that substantially reduced the amount of required SeO_2 (2 vs 11 equiv) (Scheme 1). Starting

Scheme 1



with **11**, acid-catalyzed elimination of the secondary allylic alcohol (0.6 equiv of TsOH, toluene, 110 °C, Dean–Stark trap, 2.5 h, 97%) cleanly provided the diene **12** poised for introduction of the fused unsaturated six-membered ring by a Diels–Alder reaction. Treatment of **12** with phenyl vinyl-sulfone (2 equiv, toluene, 110 °C, 45 h, 61%) provided **13** as an inconsequential mixture of cycloadduct isomers derived from β-face (top face) addition of the dienophile with installation of the C15′ stereochemistry. Mild reductive removal of the phenyl sulfone with Mg (10 equiv, MeOH, 23 °C, 4 h) and deliberate completion of the in situ methanolysis of the indole carbamate (6.5 equiv of K_2CO_3, MeOH, 23 °C, 1 h) provided **14** (65% for two steps), whose structure and stereochemistry were confirmed in a single-crystal X-ray structure determination.[22] Final reduction of the lactam **14** (5 equiv of 9-BBN, THF, 23 °C, 8 h, 59%) afforded the key substrate **6** (five steps from known intermediate **11**, eight steps from catharanthine) for coupling with vindoline.

Single-step incorporation of **6** into the targeted vinblastine **7** by room temperature Fe(III)-promoted single electron oxidative coupling with vindoline (**3**, $36/g) in aqueous 0.05 N HCl/trifluoroethanol (TFE, 10:1, 5 equiv of $FeCl_3$, 23 °C, 3

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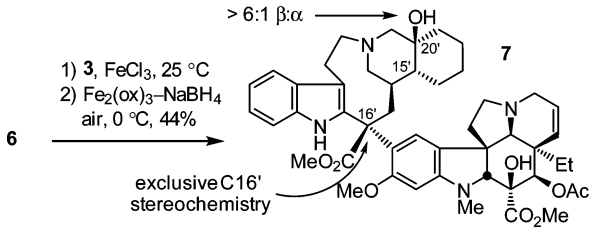
h) followed by in situ Fe(III)-promoted free radical oxidation of the trisubstituted alkene found in **8** ($Fe_2(ox)_3$, $NaBH_4$, air, 0 °C, 30 min) proceeded with complete control of the C16′ stereochemistry and good diastereoselectivity for C20′ alcohol introduction (>6:1) (Figure 3). The latter represents an



Compound	IC_{50} (nM) L1210	HCT116
7	0.6	0.7
1, vinblastine	6.0	6.8

Figure 3. Coupling of **6** with vindoline and cell growth inhibition of **7**.

increased diastereoselectivity for the C20′ alcohol introduction relative to that observed with vinblastine itself (2:1)[8] and may be attributed to the added α-face C15′ steric hindrance for reaction of O_2 with the intermediate tertiary radical derived from Fe(III)/$NaBH_4$-mediated hydrogen atom addition to intermediate **8**. Significantly, the fused six-membered ring in **6** did not impact the exclusive α-face Fe(III)-promoted coupling with vindoline.[8,23] This reaction is thought to proceed by single electron oxidation of **6** to the indole-centered radical cation and its reversible fragmentation to an intermediate charge-separated cation radical that undergoes radical addition to vindoline.[8] It is of special note that this initial coupling still occurs with exclusive control of the C16′ stereochemistry, with clean stereochemical inversion at the reacting C16′ center, despite the increased steric hindrance due to the added C15′ substitution. This observation is supportive of the suggested intermediacy of an intramolecular one-electron–two-center bonding interaction between the putative C16′ radical site and the fragmented iminium carbon that may stabilize a conformation dictating backside addition of vindoline.[8c]

Compound **7** was assessed alongside vinblastine as a direct comparison in cell growth inhibition assays against both a mouse leukemia (L1210) and human colon cancer (HCT116) cell line that have been used traditionally to initially examine vinblastine analogues (Figure 3).[24] Consistent with the design but stunning nonetheless, compound **7** proved to be 10-fold more potent than vinblastine, displaying an IC_{50} of 600–700 pM in the cell growth inhibition assays.

To confirm that the improved activity of **7** is derived from tubulin binding effects, compound **7** was compared alongside vinblastine in a tubulin binding assay, measuring their relative ability to competitively displace BODIPY–vinblastine (Figure 4).[25] Compound **7** was found to displace BODIPY–vinblastine bound to tubulin much more effectively than vinblastine itself, confirming that it binds the same site and with a much higher affinity. Although it is not possible to rule out the impact of other features,[26] this direct correlation of functional cell growth inhibition activity with target tubulin binding affinity, the relative magnitude of the effects, and the small benign structural differences between **1** and **7** suggest that the improved potency of **7** is derived predominately, if not exclusively, from on target

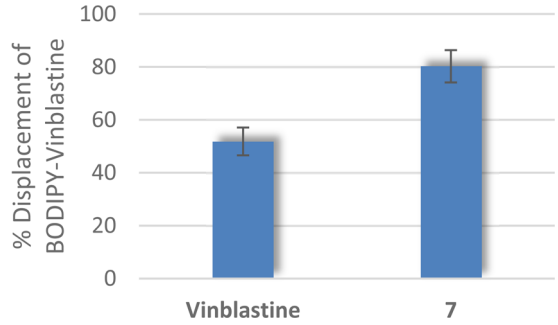


Figure 4. Tubulin (0.1 mg/mL, 0.91 μM) was incubated with BODIPY–vinblastine (BODIPY–VBL, 1.8 μM) and either vinblastine or compound **7** (18 μM) at 37 °C in PEM buffer containing 850 μM GTP. The BODIPY–VBL fluorescence intensity (FI; ex 480 nm, em 514 nm) of 100 μL aliquots from each incubation was measured in a fluorescence microplate reader at 37 °C. Control experiments were performed with BODIPY–VBL in the absence of a competitive ligand (control 1, maximum FI enhancement due to tubulin binding) and in the absence of tubulin (control 2, no FI enhancement due to tubulin binding). % BODIPY–VBL displacement was calculated by the formula (control 2 FI − experiment FI)/(control 2 FI − control 1 FI) × 100. Reported values are the average four measurements ± the standard deviation.

effects on tubulin. The modeled binding of **7** with tubulin is presented in Supporting Information Figure S1.

Approaches to improving the properties of natural products typically involve efforts to simplify or edit their structures through semisynthetic modifications, diverted total synthesis, or late-stage (C–H) functionalization. Such efforts often strive to remove, introduce, identify, or replace key functional groups to enhance target binding affinity, improve physiochemical properties, incorporate stabilizing modifications, or introduce conformational constraints. Rarely, if ever, does one consider adding benign molecular complexity to the underlying core structure.[27] In part, this may be attributed to the perceived added challenge intrinsic in the requisite compound synthesis. Herein, we describe the synthesis of a rationally designed ABC (added benign complexity)[27] modification to vinblastine that was as accessible as the natural product or its simplified analogues, enhanced the stereochemical control of the underlying synthesis, and provided an analogue 10-fold more potent than the natural product. The compound represents a vinblastine analogue accessible only by chemical synthesis and is presently inaccessible by natural product derivatization, late-stage functionalization, or biosynthetic methods. Moreover, in instances when the productive properties of a natural product are directly related to its emergence in Nature and has undergone continued optimization by natural selection as is likely the case with vinblastine, it may not be easily subjected to structural simplifications. In such cases, it is possible that added molecular complexity[15,16] may be used to enhance target binding and functional biological activity, and approaches as simple as ABC illustrated herein may represent a rational conceptual approach to achieving such objectives.[28]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b04330.

Crystal structure of **14** (CIF)

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Full experimental details and copies of ^{1}H NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Noble, R. L.; Beer, C. T.; Cutts, J. H. *Ann. N. Y. Acad. Sci.* **1958**, *76*, 882. (b) Noble, R. L. *Lloydia* **1964**, *27*, 280. (c) Review: Noble, R. L. *Biochem. Cell Biol.* **1990**, *68*, 1344.

(2) Svoboda, G. H.; Neuss, N.; Gorman, M. *J. Am. Pharm. Assoc., Sci. Ed.* **1959**, *48*, 659.

(3) For a comprehensive review of the chemistry, medicinal chemistry, biology, and clinical applications, see: (a) Kuehne, M. E.; Marko, I. Syntheses of vinblastine-type alkaloids. In *The Alkaloids*; Brossi, A., Suffness, M., Eds.; Academic: San Diego, CA, 1990; Vol. *37*, pp 77−131. (b) Borman, L. S.; Kuehne, M. E. Functional hot spot at the C-20′ position of vinblastine. In *The Alkaloids*; Brossi, A., Suffness, M., Eds.; Academic: San Diego, CA, 1990; Vol. *37*, pp 133−144. (c) Pearce, H. L. Medicinal chemistry of bisindole alkaloids. In *The Alkaloids*; Brossi, A., Suffness, M., Eds.; Academic: San Diego, CA, 1990; Vol. *37*, pp 145−204. (d) Neuss, N.; Neuss, M. N. Therapeutic use of bisindole alkaloids from *Catharanthus*. In *The Alkaloids*; Brossi, A., Suffness, M., Eds.; Academic: San Diego, CA, 1990; Vol. *37*, pp 229−240.

(4) Reviews: (a) Timasheff, S.; Andreu, J.; Gorbunoff, M.; Medranot, F.; Prakash, V. *Cell. Pharmacol.* **1993**, *1*, S27. (b) Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253.

(5) Gigant, B.; Wang, C.; Ravelli, R. B. G.; Roussi, F.; Steinmetz, M. O.; Curmi, P. A.; Sobel, A.; Knossow, M. *Nature* **2005**, *435*, 519.

(6) (a) Langlois, N.; Gueritte, F.; Langlois, Y.; Potier, P. *J. Am. Chem. Soc.* **1976**, *98*, 7017. Mangeney, P.; Zo Andriamialisoa, R.; Langlois, N.; Langlois, Y.; Potier, P. *J. Am. Chem. Soc.* **1979**, *101*, 2243. (b) Kutney, J. P.; Hibino, T.; Jahngen, E.; Okutani, T.; Ratcliffe, A. H.; Treasurywala, A. M.; Wunderly, S. *Helv. Chim. Acta* **1976**, *59*, 2858. Kutney, J. P.; Choi, L. S. L.; Nakano, J.; Tsukamoto, H.; McHugh, M.; Boulet, C. A. *Heterocycles* **1988**, *27*, 1845. (c) Kuehne, M. E.; Matson, P. A.; Bornmann, W. G. *J. Org. Chem.* **1991**, *56*, 513. Bornmann, W. G.; Kuehne, M. E. *J. Org. Chem.* **1992**, *57*, 1752. Kuehne, M. E.; Zebovitz, T. C.; Bornmann, W. G.; Marko, I. *J. Org. Chem.* **1987**, *52*, 4340. (d) Magnus, P.; Stamford, A.; Ladlow, M. *J. Am. Chem. Soc.* **1990**, *112*, 8210. Magnus, P.; Mendoza, J. S.; Stamford, A.; Ladlow, M.; Willis, P. *J. Am. Chem. Soc.* **1992**, *114*, 10232. (e) Yokoshima, S.; Ueda, T.; Kobayashi, S.; Sato, A.; Kuboyama, T.; Tokuyama, H.; Fukuyama, T. *J. Am. Chem. Soc.* **2002**, *124*, 2137.

(7) Reviews: (a) Potier, P. *J. Nat. Prod.* **1980**, *43*, 72. (b) Kutney, J. P. *Nat. Prod. Rep.* **1990**, *7*, 85.

(8) (a) Ishikawa, H.; Colby, D. A.; Boger, D. L. *J. Am. Chem. Soc.* **2008**, *130*, 420. (b) Ishikawa, H.; Colby, D. A.; Seto, S.; Va, P.; Tam, A.; Kakei, H.; Rayl, T. J.; Hwang, I.; Boger, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 4904. (c) Gotoh, H.; Sears, J. E.; Eschenmoser, A.; Boger, D. L. *J. Am. Chem. Soc.* **2012**, *134*, 13240. (d) Leggans, E. K.; Barker, T. J.; Duncan, K. K.; Boger, D. L. *Org. Lett.* **2012**, *14*, 1428.

(9) Sears, J. E.; Boger, D. L. *Acc. Chem. Res.* **2015**, *48*, 653.

(10) Sasaki, Y.; Kato, D.; Boger, D. L. *J. Am. Chem. Soc.* **2010**, *132*, 13533.

(11) Tam, A.; Gotoh, H.; Robertson, W. M.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6408.

(12) Va, P.; Campbell, E. L.; Robertson, W. M.; Boger, D. L. *J. Am. Chem. Soc.* **2010**, *132*, 8489.

(13) Schleicher, K. D.; Sasaki, Y.; Tam, A.; Kato, D.; Duncan, K. K.; Boger, D. L. *J. Med. Chem.* **2013**, *56*, 483.

(14) Sears, J. E.; Barker, T. J.; Boger, D. L. *Org. Lett.* **2015**, *17*, 5460.

(15) Gotoh, H.; Duncan, K. K.; Robertson, W. M.; Boger, D. L. *ACS Med. Chem. Lett.* **2011**, *2*, 948.

(16) (a) Leggans, E. K.; Duncan, K. K.; Barker, T. J.; Schleicher, K. D.; Boger, D. L. *J. Med. Chem.* **2013**, *56*, 628. (b) Barker, T. J.; Duncan, K. K.; Otrubova, K.; Boger, D. L. *ACS Med. Chem. Lett.* **2013**, *4*, 985.

(17) Wilson, R. M.; Danishefsky, S. J. *J. Org. Chem.* **2006**, *71*, 8329.

(18) Reviews: (a) Szpilman, A. M.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 9592. (b) Hong, J. *Chem. - Eur. J.* **2014**, *20*, 10204.

(19) (a) Martin, S. F.; Clements, J. H. *Annu. Rev. Biochem.* **2013**, *82*, 267. (b) Larsen, E. M.; Wilson, M. R.; Taylor, R. E. *Nat. Prod. Rep.* **2015**, *32*, 1183.

(20) (a) Boger, D. L.; Brotherton, C. E. *J. Org. Chem.* **1984**, *49*, 4050. (b) Boger, D. L.; Mullican, M. D. *J. Org. Chem.* **1984**, *49*, 4033. (c) Boger, D. L.; Mullican, M. D. *J. Org. Chem.* **1984**, *49*, 4045.

(21) Giovanelli, E.; Leroux, S.; Moisan, L.; Carreyre, H.; Thuery, P.; Buisson, D.-A.; Meddour, A.; Coustard, J.-M.; Thibaudeau, S.; Rousseau, B.; Nicolas, M.; Hellier, P.; Doris, E. *Org. Lett.* **2011**, *13*, 4116.

(22) The structure of **14** was confirmed in a single-crystal X-ray structure determination conducted on colorless rods grown from CH_2Cl_2 and has been deposited with the Cambridge Crystallographic Data Center (CCDC 1475224).

(23) (a) Vukovic, J.; Goodbody, A. E.; Kutney, J. P.; Misawa, M. *Tetrahedron* **1988**, *44*, 325. (b) Szantay, C., Jr.; Balazs, J.; Bolcskei, J.; Szantay, C. *Tetrahedron* **1991**, *47*, 1265. (c) Sundberg, R. J.; Hong, J.; Smith, S. Q.; Sabat, M.; Tabakovic, I. *Tetrahedron* **1998**, *54*, 6259.

(24) Kuehne, M. E.; Bornmann, W. G.; Marko, I.; Qin, Y.; Le Boulluec, K. L.; Frasier, D. A.; Xu, F.; Mulamba, T.; Ensinger, C. L.; Borman, L. S.; Huot, A. E.; Exon, C.; Bizzarro, F. T.; Cheung, J. B.; Bane, S. L. *Org. Biomol. Chem.* **2003**, *1*, 2120.

(25) (a) Jiang, J. D.; Davis, A. S.; Middleton, K.; Ling, Y. H.; Perez-Soler, R.; Holland, J. F.; Bekesi, J. G. *Cancer Res.* **1998**, *58*, 5389. (b) Zhang, C.; Yang, N.; Yang, C.; Ding, H.; Luo, C.; Zhang, Y.; Wu, M.; Zhang, X.; Shen, X.; Jiang, H.; et al. *PLoS One* **2009**, *4*, e4881. (c) Rai, A.; Surolia, A.; Panda, D. *PLoS One* **2012**, *7*, e44311. (d) Rashid, A.; Kuppa, A.; Kunwar, A.; Panda, D. *Biochemistry* **2015**, *54*, 2149.

(26) cLogP 5.23 versus 5.61 for **1** and **7**, polar surface area unchanged (ChemBio3D Ultra).

(27) The target molecule (analog) is intrinsically more complex than the natural product (added complexity), and the change is benign in the sense that it does not alter the existing functionality, perturb the conformational properties, or add new functional groups to the natural product. Herein, this arises through an alteration in the core structure of the natural product.

(28) Taken from ref 18a and in the words of Leonardo da Vinci: "Where nature finishes producing its own species, man begins, using natural things and in harmony with this very nature, to create an infinity of species."

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